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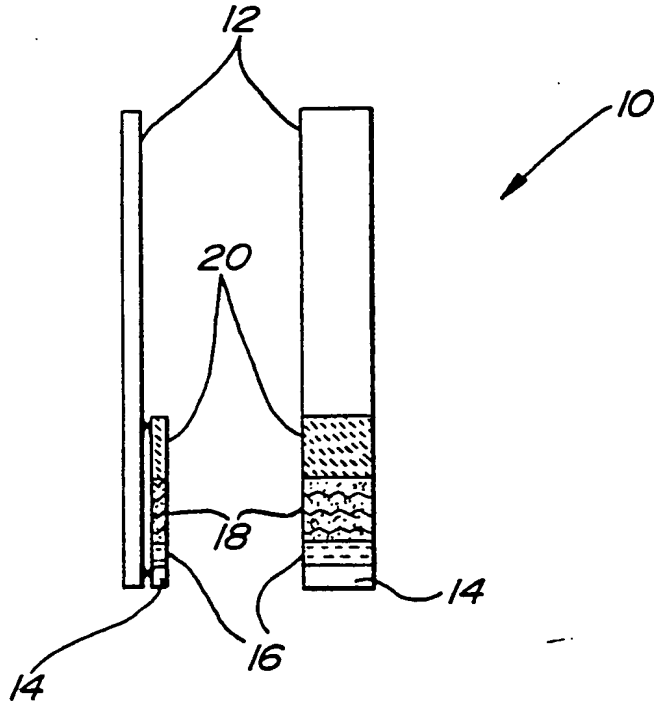
(54) Title: BIOANALYTICAL DETECTION SYSTEM

(57) Abstract

A device and process for detecting the presence of organic molecular analytes in a fluid comprising a first binding (18) component having a predetermined first affinity for specifically reversibly binding an analyte, a molecular conjugate of the analyte with a signal generating molecule that generates a detectable signal, and a second binding (20) component having a predetermined second affinity for reversibly binding the signal generating molecule. In the presence of analyte, a fluid conducting system allows competitive binding of the analyte with the molecular conjugate and causes displacement of the conjugate and conducts the displaced conjugate to the second binding component. The signal generating molecule generates a detectable signal distinguishing binding thereof at the first or second binding components thereby indicating the presence of the analyte in the fluid.



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# BIOANALYTICAL DETECTION SYSTEM

## TECHNICAL FIELD

This invention relates to a device and process for detecting the presence of organic molecular analytes in a fluid. More specifically, the invention relates to a device and process for using either a discontinuous, single use disposable analytical device or a continuous use device for continually measuring concentration changes of substances such as hormones, drugs, or various other biologically active substances in the submicrogram range with a specificity comparable to radioimmuno assay.

## BACKGROUND OF THE INVENTION

Various methods have been derived for monitoring low molecular weight compounds in blood plasma or other biological media. For example, enzyme electrodes have been used for the direct measurement of biomolecules in physiological samples, such as glucose, urea, amino acids, and others. These enzyme electrodes include a selective enzyme layer immobilized at the surface of a potentiometer or amperometeric device that senses the steady state concentration of a product formed in the immobilized layer as the substrate for the enzyme diffuses into this reactive film.

The U.S. Patent 4,344,438 to Schultz, issued August 17, 1982 discloses an optical sensor for monitoring low molecular weight compounds in blood plasma. The device utilizes a binding protein

immobilized inside a membrane. A conjugate between glucose and fluorescein serves to generate a signal. The conjugate binds to an immobilized lectin in the absence of glucose such that there is no fluorescence in an adjacent dialysis chamber. If glucose enters into the lumen through the membrane, it competes with binding sites on the lectin and sets free glucose fluorescein conjugate, hence there is fluorescence in the detector tube. It is disadvantageous to use enzymes as signal generators in this system because the dissociated conjugate in the presence of glucose in the middle of the detector tube could provide a high background signal so that the reduced amount of conjugate on the side of the tube might not be distinguishable from the background or from the previous signal where no external glucose was present.

The U.S. Patent 4,517,288 to Giegel et al, issued May 14, 1985 discloses a method for conducting a ligand assay in an inert porous medium wherein a binding material is immobilized within the medium. The method includes the steps of immobilizing a binding material within a finite zone of a medium and applying an analyte to the zone containing the immobilized binding material. A labeled indicator is applied to the zone and becomes bound within the zone in an amount which can be correlated to the amount of the analyte in the zone. A solvent is applied to the center of the zone to chromatographically separate the unbound labeled indicator from the zone and measuring the amount of labeled indicator remaining in the zone. This chromatographic method cannot be used in a continuous assay device for continually determining changes in a dynamic system.

Anderson et al reported in Clin. Chem. 34/7, 1417-1421 (1988) of a fiberoptic chemical sensor for continuous, reversible measurement of phenytoin. Beta-phycoerythrin-phenytoin and Texas Red-labeled antibody to phenytoin were sealed inside a short length of cellulose dialysis tubing. The tubing was cemented to the distal end of an optical fiber. When the sensor was alternately placed into solutions with various concentrations of free phenytoin, the drug crossed the dialysis membrane and displaced a fraction of the beta-phycor eythrin-phenytoin from the antibody. The resulting change in fluorescent signal was measured with a fiberoptic fluorometer. A reversible sensor could be made that has a response time suitable for continuous measurements utilizing the invention. In an abstract presented at the proceedings of the symposium on Sensor Science and Technology, April 6 - 8, 1987 and published in the same proceeding abstracts by the Electrochemical Society, Inc. of Pennington, New Jersey, Vol. 87-15, W. Schramm, et al (the inventor of the present application) postulated an immunoglobulin-based biosensor including heterobifunctional structures that bind reversibly to immobilized counterparts. A combination of two antibody-antigen reactions hypothetically would generate a signal for continuous monitoring of analytes. The abstract hypothesizes several biosensor systems, the abstract being published prior to any attempt at reduction to practice of the concept.

The present invention provides means for constructing either a discontinuous single use disposable analytical test device or a reversible

biosensor for continuous measurements of analytes. The invention utilizes the concept of a dislocated conjugate, such as the general concept being disclosed in the Shultz patent, but goes further so as to bind the conjugate at another site and measure it at that second site. Accordingly, the present invention can utilize enzymes as a signal generator. Further, the present invention can generate two signals, one at each of the two binding sites.

## SUMMARY OF THE INVENTION

In accordance with the present invention, there is provided a device for detecting the presence of organic molecular analytes in fluid, the device including first binding means having a predetermined first affinity for specifically, reversibly binding the analyte, and a molecular conjugate of the analyte with a signal generating molecule which generates a detectable signal. Second binding means has a predetermined second affinity for reversibly binding the signal generating molecule. Fluid conducting means conducts the fluid to the first binding means allowing competitive binding of the analyte in the fluid and displacement of the conjugate and for conducting the displaced conjugate to the second binding means. The signal generator molecule generates a detectable signal distinguishing binding thereof at the first or second binding means thereby indicating the presence of analyte in the fluid.

The present invention further provides a process for detecting the presence of organic molecular analytes in the fluid, the method including the steps of binding the molecular conjugate of the

analyte coupled to the signal generating molecule, which generates the detectable signal, to a first binding molecule having a predetermined first affinity for specifically reversibly binding the analyte and conducting the fluid to the first binding molecule and competitively binding analyte in the fluid and displacing bound conjugate. The displaced conjugate is conducted to a second binding molecule having a second affinity for reversibly binding the signal generating molecule. A detectable signal is generated which distinguishes binding of the conjugate at the first or second binding molecules thereby indicating the presence of analyte in the fluid.

## FIGURES IN THE DRAWINGS

Other advantages of the present invention will be readily appreciated as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings wherein:

Figure 1 is a schematic representation of a first embodiment of the present invention shown in plan and side views;

Figure 2 is a schematic representation of the present invention illustrating the function of each component of the first embodiment of the invention;

Figure 3 is a schematic representation of the operation of the first embodiment of the present invention;

Figure 4 is a schematic representation of a second embodiment of the present invention and its operation, showing enlarged views;

Figure 5 is a dose response curve of dissociated hetrobifunctional conjugate from the immobilized antibody to progestrone (full circles), and associated conjugate on the immobilized antibody to horseradish to peroxidase (open circles) in the presence of increasing amounts of progestrone (x-axis); and

Figure 6 is a chart showing the signals of the dose response curves from Figure 10 added and the lowest value set to zero to obtain an additive standard curve.

## DETAILED DESCRIPTION OF THE DRAWINGS

A device for detecting the presence of organic molecular analytes in a fluid is generally shown at 10 in the Figures. Primed numerals are used to indicate similar structures between the several embodiments.

The term analyte is used herein to mean any molecular species within a body fluid, such as progesterone, testosterone, and benzoylecgonine. Conceivably, the present invention could also be used for the determination of other organic and inorganic species of molecules present in various fluids which are not necessarily body fluids or organic fluids.

Generally, the invention comprises the combination of first binding means having a first affinity for specifically reversibly binding the analyte, a molecular conjugate of the analyte with a signal generating molecule, referred to as a

heterobifunctional complex, second binding means having a second affinity for reversibly binding the signal generating molecule, and a fluid conducting mechanism for conducting the fluid to the first binding means allowing competitive binding of the analyte in the fluid and displacement of the conjugate from the first binding means and for conducting the displaced conjugate to the second binding means. The signal generator molecule generates a detectable signal distinguishing binding thereof at the first or second binding means thereby indicating the presence of analyte in the fluid. The invention thereby provides a shuttle signal generating mechanism capable of qualitatively or quantitatively measuring analyte in the fluid.

The ratio of binding affinity of the first binding component and second the binding component to their respective counterpart of the molecular conjugate, or the ratio of concentrations of the first binding component and the second binding component are adjusted such that in the absence of analyte, the molecular conjugate is predominately bound to the first binding component. In this state, a signal is generated by means of the molecular conjugate on the first binding component.

A first embodiment of the invention is generally shown at 10 in Figure 1. This embodiment of the invention is a disposable test strip for the detection of complex organic molecules in liquids. The presence of the analyte in the liquid sample is indicated on the test strip 10 by a signal, such as a color, at a distinct location on the test strip 10.

The absence of the molecule is indicated by a signal at a different distinct location on the test strip 10.

More specifically, the test strip 10 includes a support strip 12. The support strip 12 can be made from a polymer, such as Mylar®. Four absorbent membranes 14,16,18,20 are attached to the support polymer 12. The absorbent membranes 14,16,18,20 are attached to the polymer strip 12 so as to be adjacent to each other.

Absorbent membrane 14 provides a conducting means for transporting medium by capillary action to membrane 16. Membrane 16 is capable of conducting the medium by capillary action to membrane 18, and membrane 18 is capable of transporting the medium by capillary action to membrane 20. Accordingly, the membranes 14,16,18,20 function as the fluid conducting means providing separate adjacent zones to each other.

The first binding means is immobilized, chemically or physcially, on absorbent membrane 18. This first binding means specifically binds the analyte to be measured. The second binding means is immobilized on membrane 20. The first and second binding means function as bioreceptors for specifically binding either the analyte or signal generator. The first and second binding means can be selected from the group consisting essentially of lectins, receptors, membrane proteins, transport proteins, ribonucleic acid, complimentary subunits, monoclonal and polyclonal antibodies, and other compounds that selectively and competitively bind to the analyte or signal generator, respectively. The first and second binding means can be a proteinacious

or nonproteinacious nature. The lectins, receptors, and proteins described above are proteinacious binding means. Examples of nonproteinacious reversible binders can be chelating ligands, complexing agents, or charged ligands.

The second binding means is immobilized, either physically or chemically bound, to absorbent membrane 20.

The signal generating means can be selected from a group consisting essentially of enzymes, fluorescent molecules, ultraviolet absorbent agents and other compounds capable of conjugation with the analyte without deletion of the capacity to generate the signal. Accordingly, the second binding means can be a bioreceptor that specifically and reversibly binds the signal generating compound. For example, the membrane 20 may have a monoclonal antibody that binds specifically to a signal generator, such as an enzyme.

The absorbent membrane 16 is impregnated with the conjugate such that the conjugate easily migrates with a water front from the end of the strip 10 as the end of strip 10 is immersed in an aqueous medium. If the signal generating means is an enzyme, the conjugate or heterobifunctional reagent comprises an enzyme covalently bound to the analyte. The enzyme in the conjugate converts a substrate into a product such that the product is distinctively different from the substrate. The product can be measured, such as when the enzyme catalytically converts a colorless substrate into a colored product.

The function of each component of the invention is illustrated in Figure 2. Box A illustrates the second binding means or receptor 22 covalently bound to the membrane 20. The binding molecule 22 is capable of specifically and reversibly binding enzyme 24. Box B in Figure 2 illustrates the first binding means as specific binding molecule 26 covalently bound to membrane 18, the molecule 26 specifically and reversibly binding analyte 28. Box C schematically illustrates the conjugate 30 of the analyte 29 bound to the enzyme 24. Analyte 29 corresponds structurally and functionally to unbound analyte 28. Box D illustrates the conversion of substrate (S) to product (P) by the enzyme component 24 of the conjugate 30.

The operation of the invention is schematically shown in Figure 3. The test strip 10 is dipped into a specimen solution 32 as schematically shown in Figure 3A. The specimen solution is absorbed into the first membrane 14 and carried sequentially through membranes 16,18,20, the solvent front being indicated in the Figures at 34. Each Figure 3A through C shows the test strip 10 and an enlarged portion of the strip illustrating the detection process.

If no external analyte is present in the specimen solution 32, the conjugate 30 binds to the immobilized binding molecule on the absorbent membrane 18, developing a color on this membrane. This indicates the absence of analyte in the specimen solution 32. Such binding is shown in Figure 3B. The conjugate 30 will preferentially bind to the binding molecule 26 immobilized on membrane 18 because of the affinity of the binding molecule 26

for the analyte portion 29 of the conjugate 30. Since there is no other competitive binding for the binding molecule 26, the conjugate 30 will bind thereto.

In the presence of free analyte 28 in the specimen solution, the free analyte 28 competes for binding sites with the bound analyte 29 of the conjugate 30 on the binding molecule 26 immobilized at membrane 18. The conjugate 30 diffuses to membrane 20 where it binds to the antibody specifically recognizing the signal generating enzyme 24. Upon exposure to substrate solution, the enzyme 24 develops color on membrane 20 which indicates the presence of free analyte 28 in the specimen solution. In other words, the conjugate 30 either remains on membrane 18, where it is bound, thereby indicating the absence of free analyte, or free analyte competitively binds with the conjugate 30 for binding sites at membrane 18. In this case, the conjugate 30 will not bind totally at membrane 18 and be carried to membrane 20. The conjugate 30 will then bind at membrane 20. Upon being bound at membrane 20, the conjugate 30 is capable of converting substrate to a colored product at membrane 20 thereby indicating the presence of free analyte 28 in the solution.

A second embodiment of the invention is schematically shown in Figure 4. Rod 34 contains the binding means 26' covalently bound thereto. Rod 36 includes receptor 22' covalently bound thereto. Each of the rods 34,36 can be made from polymers, plastic sheets, glass slides, ceramic, silicon chips, and the like. Each of Figures 4A through C illustrate the

subject process utilizing the rods 34,36 and further show an enlargement illustrating the invention at the molecular level.

The rods 34,36 are immersed in a tube 38 containing a liquid sample specimen 32' and the heterobifunctional binder 30'. The rods 34,36 are constructed such that the heterobifunctional binder 30' binds predominately only to the binding means 26' (first binding means) recognizing specifically the bound analyte 29' if no external free analyte 28' is present in the sample specimen 32'.

If the rods 34,36 are removed from the specimen sample 32' and exposed to a solution containing substrate (the reaction being illustrated in Figure 2D), a distinctively measurable product will be detectable and developed on the left rod 34, because the conjugate 30' will bind preferentially to the left rod 34 as opposed to the right rod 36. This is because, as explained above, the totality of bioreceptor 26' on the rod 34 has a greater binding affinity for the analyte portion 29' of the conjugate 30' then the binding means 22' has for the enzyme portion 24' of the conjugate 30'. As discussed above, this may be because of a greater binding affinity per binding means molecule or because of a greater amount of binding means 26' on rod 34 as opposed to binding means 22' on rod 36. Color development on rod 34 thereby indicates that the sample does not contain analyte 28'.

If the sample 32' contains free analyte 28', the free analyte molecules 28' compete for binding sites on the immobilized binding means 26' for the bound analyte 29' on the heterobifunctional binder 30'. The heterobifunctional binder 30'

diffuses to the opposite rod 36 and is there eventually bound to the binding means 22' specifically recognizing the signal generating enzyme portion 24' of the heterobifunctional binder 30'.

If the two rods 34,36 are now exposed to a solution containing substrate, only the rod 36 containing the binding means 22' to the signal generator portion 24' of the conjugate 30' develops a distinctively measurable product, such as a color. This indicates that the sample does contain the free analyte 28'.

EXAMPLE

This combination of two antibodies as binders and a heterobifunctional conjugate between a steroid and an enzyme as signal generator has been used for the quantitative determination of progesterone by colorimetric detection. A monoclonal antibody to progesterone was immobilized to the surface of a polyacrylamide rod. Another monoclonal antibody, specifically recognizing horseradish peroxidase (HRP), was immobilized to a polystyrene test tube. The conjugate consists of progesterone-horseradish peroxidase.

Immobilization of antibodies. Monoclonal antibodies of the subtype $IgG_{2bk}$ were purified by ammonium sulphate precipitation and subsequent affinity chromatography on Protein-A immobilized to cyanogen bromide activated agarose. The immunoglobulin was eluted from the Protein-A column by dissociation with acetic acid, pH 3.5, and dialyzed against phosphate buffer, 0.01 mol/L, pH 7.4. The concentration of the antibody was adjusted to 1 mg/mL by UV-spectroscopy at 280 nm absorbance.

The polymer surfaces (polystyrene and polyacrylamide) were coated for 1 h at room temperature with avidin, 1ug/mL in phosphate buffer in the presence of 1g/L of glutardialdehyde. The surfaces were washed with de-ionized water containing 1g/L triton X and subsequently incubated with a solution of 10 and 100 ug/mL of antibody (anti-progesterone and anti-HRP antibody, respectively) in phophate buffer. The remaining aldehyde functions on the cross-linked avidin were sufficient to bind the immunoglobulins covalently. The surfaces with immobilized antibodies were washed with phosphate buffer containing 1 g/L Trition X and 1 mmol/L sodium cyanoborohydrate. The monoclonal antibody to horseradish peroxidase was immobilized to the polystyrene tube (6mm ID; Skatron Inc., Sterling, VA), the monoclonal antibody to progesterone was immobilized to the polyacrylamide rod (4.4 mm OD).

Progesterone-horseradish peroxidase conjugate. Progesterone 11a-hemisuccinate (14 umol) was dissolved in 0.5 ml of dimethylformamide and 15 umol of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride in 200 uL of dioxane was added and stirred for 20 min at room temperature. The reaction mixture was added to a solution of 30 umol of spermidine (free base) in 300 uL of dioxane and incubated overnight at room temperature . After adding 3 mL of chloroform, the steroid derivative was separated from by-products by extraction with water and recovered from the chloroform phase. The preparation which was recovered by evaporation of chloroform with nitrogen was used for the reaction with horseradish peroxidase (HRP).

HRP (100 nmol) was dissolved in 400 uL of de-ionized water and 200 uL of a solution of sodium periodate in water (0.1 mol/L was added and the solution stirred for 20 min at room temperature. The progesterone derivative was dissolved in 600 uL of dimethyl formamide and slowly added under agitation to the oxidized HRP. The acidity of the solution was raised with sodium carbonate buffer, 0.5 mol/L, to pH 9.5 and 20 uL of sodium cyanoborohydride, 1 mol/L, was added. The reaction mixture was stirred for 2 hours at room temperature and dialyzed against six changes of a total of 4 L phosphate buffer, 0.01 mol/L. The dialyzed solution was purified over a cross-linked polyacrylamide gel exclusion column, P-30 (BioRad, Richmond, CA) with a gel volume of 10 mL.

The prepurified HRP-progesterone derivative was finally purified by affinity chromatography. A monoclonal antibody with low affinity to the steroid ($K_a=7X10^8$ L/mol) was immobilized on cyanobromide activated agarose (5% cross-linked). HRP-progesterone conjugate applied to this gel was eluted with phosphate buffer. Nonreacted enzyme eluted first and was separated from ligand-enzyme conjugate.

Quantitative determination of progesterone. Into each test tube containing a rod, $5x10^{-14}$ mol/Lof HRP-progesterone conjugate in 145 uL was added. Thereafter, increasing amounts of progesterone from $1.25x10^{-11}$g (12.5pg/tube) to $11x10^{-8}$g (10ng/tube) were added in a volume of 5 uL. The solutions were incubated for 90 minutes, the tubes with the rods washed with de-ionized water, separated, and incubated in reagent solution for color development.

HRP was quantitatively determined by oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) and colorimetric detection of the oxidized product at 410 nm. For 100 mL of TMB reagent, 1 mL of a stock solution of 10 mg/mL TMB in dimethyl sulfoxide and 10 uL of a 30% solution of hydrogen peroxide was added citric acid, 0.05 mol/L, pH 4.5. The developing color was stopped after 20 min with sulfuric acid, 1 mol/L, and the absorbance monitored at 600 nm.

Figure 5 shows the dose-response curve for the correlation between progesterone added (abscissa) vs. the amount of heterobifunctional conjugate bound to the solid-phase with the progesterone antibody (full circles) and the HRP antibody (open circles). The additive signals (i.e. subtracting the signal for the HRP-antibody matrix form the signal of the progesterone-antibody matrix at a given progesterone concentration and setting the lowest value to zero) are shown in Figure 6.

What is claimed is:

1. A device for detecting the presence of molecular analytes in a fluid, said device comprising: first binding means having a predetermined first effective affinity for specifically reversibly binding the analyte; a molecular conjugate of the analyte with a signal generating molecule that generates a detectable signal; second binding means having a predetermined second effective affinity for reversibly binding said signal generating molecule; and fluid conducting means for conducting the fluid to the first binding means allowing competitive binding of the analyte in the fluid and displacement of said conjugate and for conducting said displaced conjugate to said second binding means, said signal generator molecule generating a detectable signal distinguishing binding thereof at said first or second binding means thereby indicating the presence of analyte in the fluid.

2. A device as set forth in claim 1 wherein said first and second binding means are selected from the group consisting essentially of lectins, receptors, membrane proteins, transport proteins, ribonucleic acid, complementary subunits, monoclonal and polyclonal antibodies, and other compounds that selectively and competitively bind the analyte for signal generation, respectively.

3. A device as set forth in claim 2 wherein said signal generating means are selected from a group consisting essentially of enzymes, fluorescent molecules, ultraviolet absorbent agents

and other compounds capable of conjugation with said analyte without deletion of the capacity to generate the signal.

4. A device as set forth in claim 3 including detector means for detecting the signal generated by said signal generating molecule.

5. A device as set forth in claim 4 wherein said signal generating means is an enzyme for metabolizing a substrate into a product, said detecting means including a first electrode adjacent said first binding means and a second electrode adjacent said second binding means for detecting the potential difference therebetween as changed by the amount of electrolyte generated proximate thereto.

6. A device as set forth in claim 3 including a support surface, said first and second binding proteins being immobilized to said support surface.

7. A device as set forth in claim 6 wherein said first binding means is immobilized on said support surface at a first zone, said second binding means being immobilized on said support surface at a second zone adjacent said first zone, and a predetermined amount of said conjugate is supported on said support surface at a third zone adjacent said first zone, said first zone being disposed between said second and third zones, said conducting means conducting fluid sequentially over said third zone carrying said conjugate therefrom to said first zone where the analyte in the fluid

competes for binding to said first binding means, said conducting means conducting the fluid containing the remaining unbound conjugate to said second binding means whereby signal generated from said conjugate at said second binding means indicates the presence of analyte in the fluid.

8. A device as set forth in claim 7 wherein said conducting means includes an absorbent membrane at each of said zones on said support surface, said first and second binding means being immobilized on said membrane at said first and second zone, respectively and said conjugate impregnating said membrane at said third zone.

9. A process of detecting the presence of organic molecular analytes in a fluid, said method including the steps of: reversibly binding a molecular conjugate of the analyte and a signal generating molecule which generates a detectable signal to a first binding molecule having a predetermined first effective affinity for specifically reversibly binding the analyte; conducting the fluid to the first binding molecule and competitively binding analyte in the fluid and displacing bound conjugate; conducting the displaced conjugate to a second binding molecule having a second effective affinity for reversibly binding the signal generating molecule and binding the conjugate to the second binding molecule; and generating a detectable signal distinguishing binding of the conjugate at the first or second binding molecules thereby indicating the presence of analyte in the fluid.

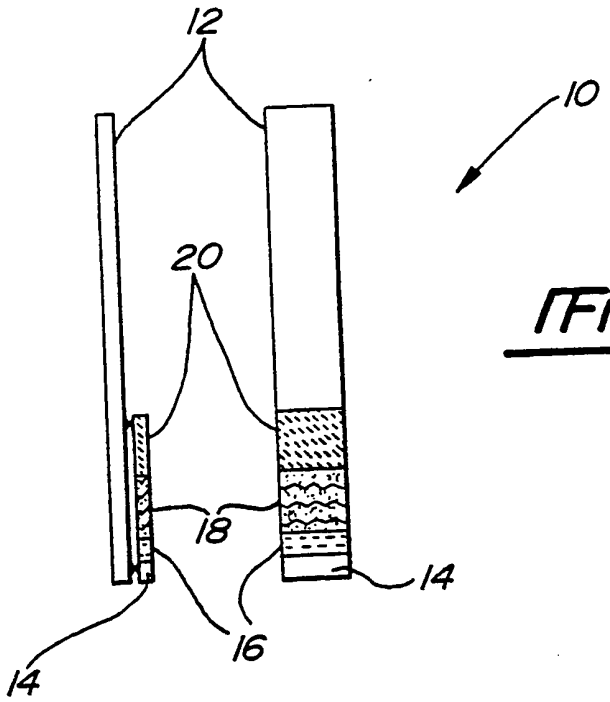
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Fig-1

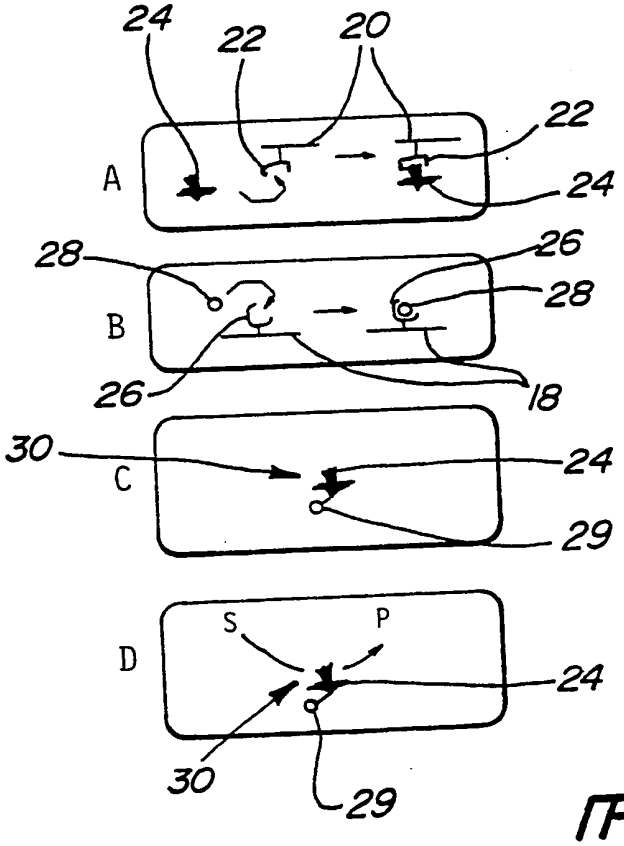
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Fig-2

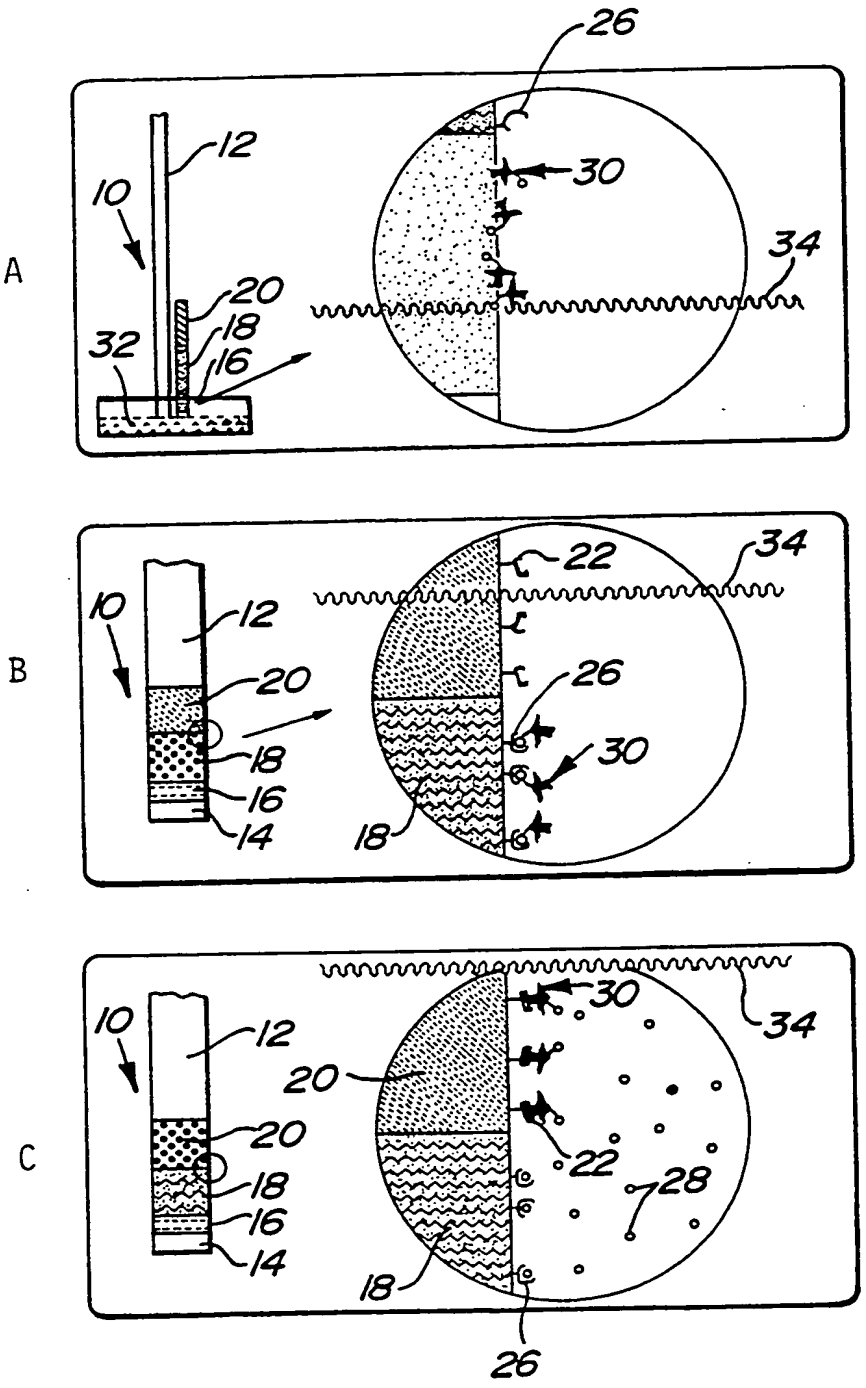


Fig-3

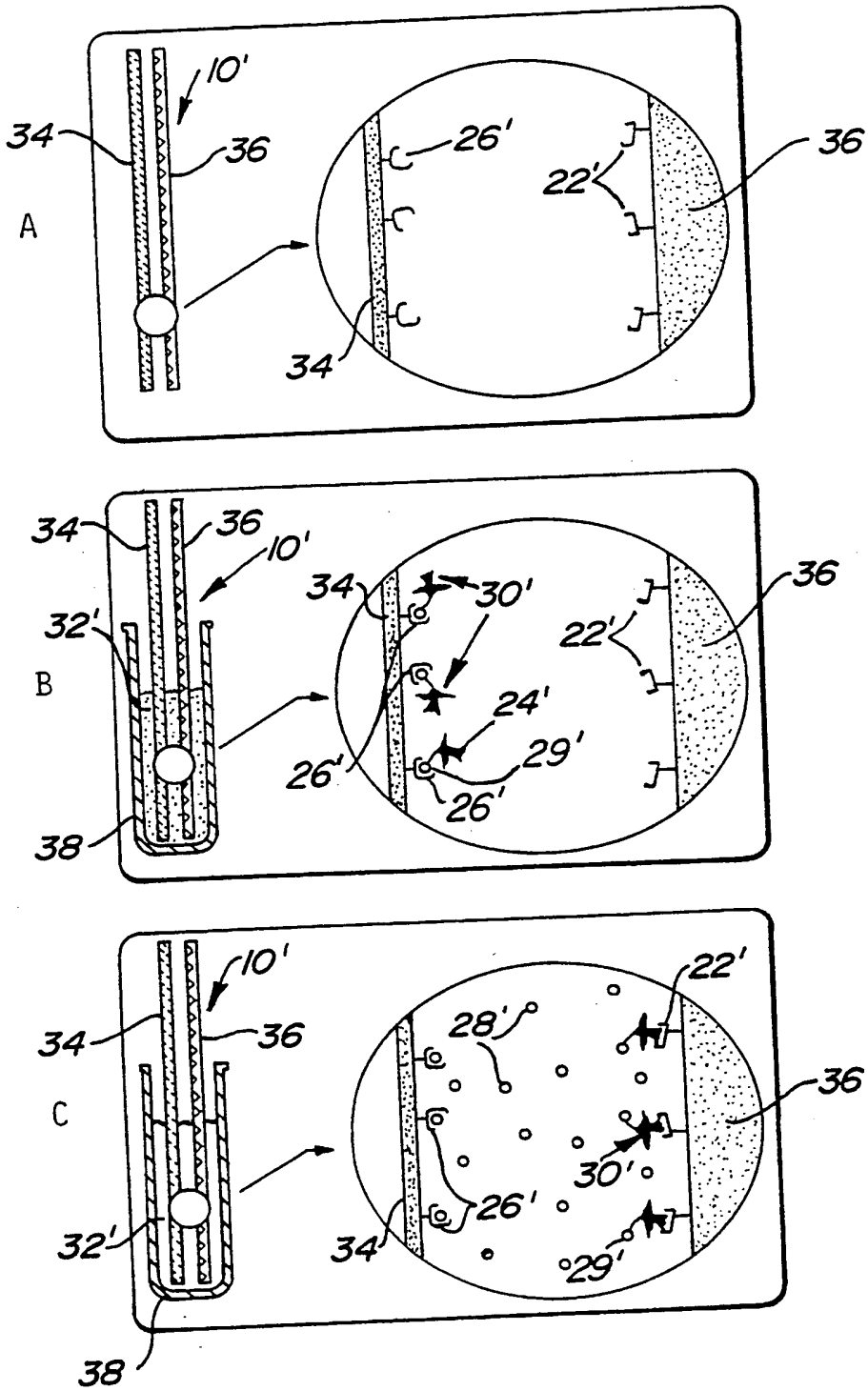
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Fig-4

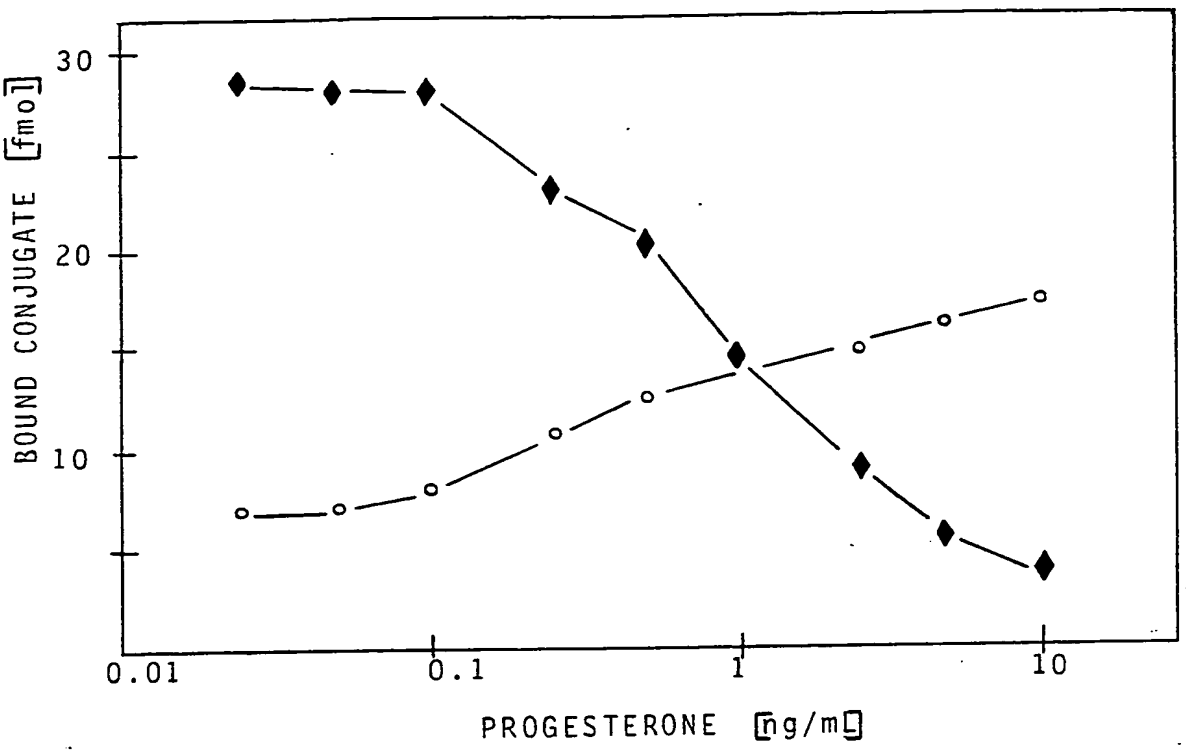


Fig-5

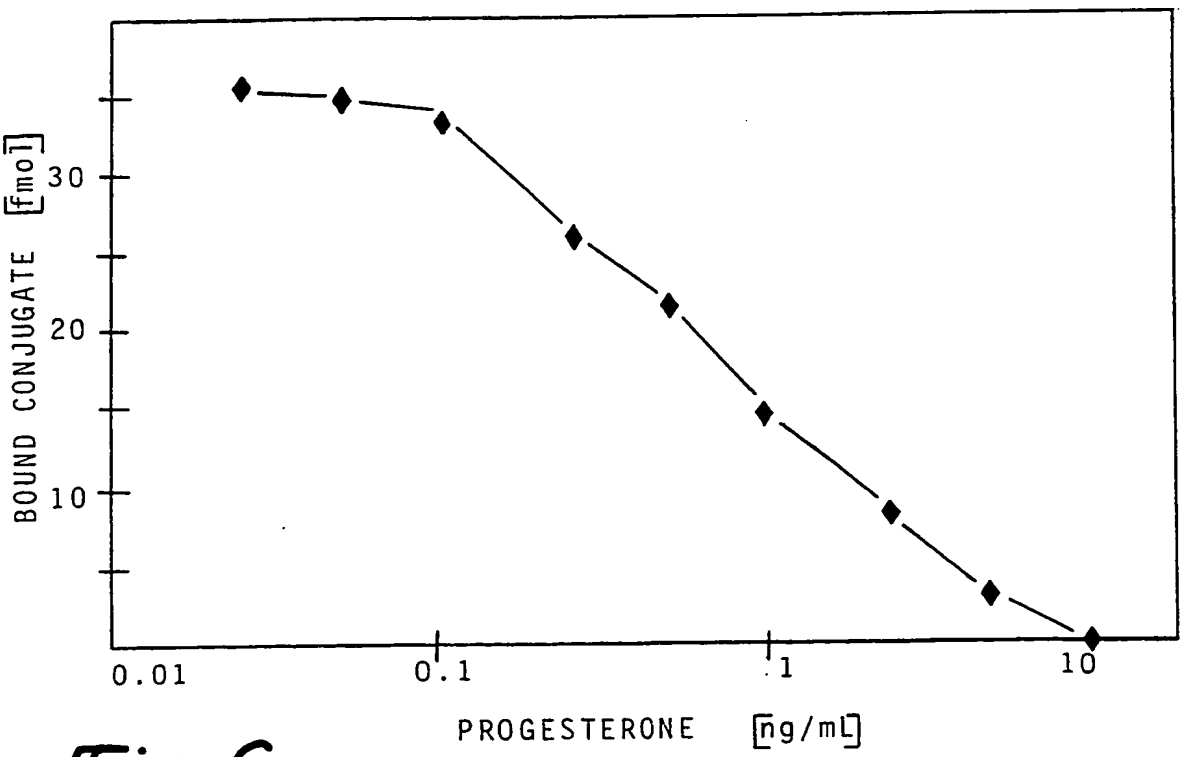


Fig-6

# INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/05511

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): G01N 33/543; 35/535
US. Cl: 436/518; 435/7.1

## II. FIELDS SEARCHED

Minimum Documentation Searched [4]

| Classification System [5] | Classification Symbols |
|---|---|
| US. CL: | 435/7.1, 288, 805, 810; 436/518, 514, 544 546, 806, 807, 808, 809, 819; 422/56, 57, 58, 61 |

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [6]

## III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

| Category * | Citation of Document, [16] with indication, where appropriate, of the relevant passages [17] | Relevant to Claim No. [18] |
|---|---|---|
| X<br>Y | US, A, 4,803,170 (STANTON et al.) 07 February 1989, see the abstract and columns 3, 5 and 7. | 1-4 & 6-9<br>5 |
| Y | US, A, 4,277,560 (GRAY et al.) 07 July 1981, see the abstract. | 5 |

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## IV. CERTIFICATION

| Date of the Actual Completion of the International Search [2] | Date of Mailing of this International Search Report [2] |
|---|---|
| 04 JANUARY 1991 | 06FEB 1991 |
| **International Searching Authority [1]** | **Signature of Authorized Officer [20]** |
| ISA/JS | Toni R. Scheiner<br>Toni R. Scheiner |

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# INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent)*, DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

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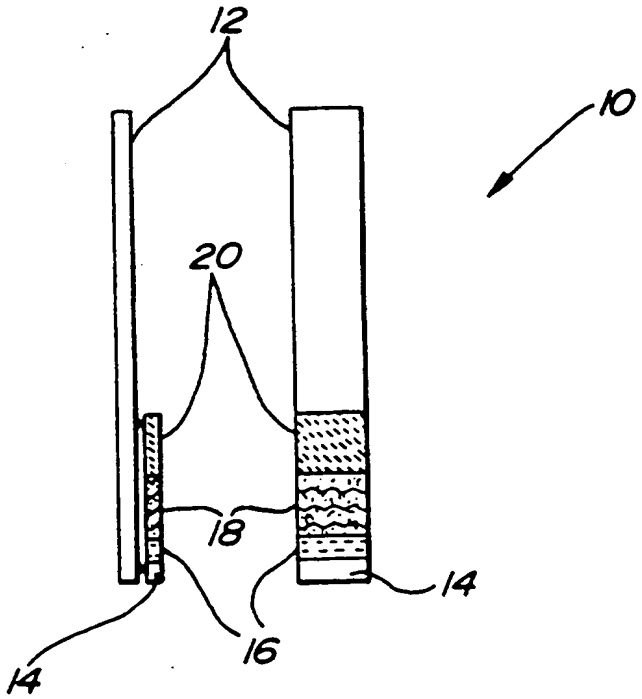
9 July 1992 (09.07.92)

## (54) Title: BIOANALYTICAL DETECTION SYSTEM

(57) Abstract

A device and process for detecting the presence of organic molecular analytes in a fluid comprising a first binding (18) component having a predetermined first affinity for specifically reversibly binding an analyte, a molecular conjugate of the analyte with a signal generating molecule that generates a detectable signal, and a second binding (20) component having a predetermined second affinity for reversibly binding the signal generating molecule. In the presence of analyte, a fluid conducting system allows competitive binding of the analyte with the molecular conjugate and causes displacement of the conjugate and conducts the displaced conjugate to the second binding component. The signal generating molecule generates a detectable signal distinguishing binding thereof at the first or second binding components thereby indicating the presence of the analyte in the fluid.




** (Referred to in PCT Gazette No. 17/1992, Section II)

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**Until further notice, any designation of "DE" in any international application whose international filing date is prior to October 3, 1990, shall have effect in the territory of the Federal Republic of Germany with the exception of the territory of the former German Democratic Republic.**

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# INTERNATIONAL SEARCH REPORT

corrected US 3/06/91

International Application No PCT/US90/05511

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): G01N 33/543
US. Cl: 436/518; 435/7.1

## II. FIELDS SEARCHED

Minimum Documentation Searched [4]

| Classification System [5] | Classification Symbols |
|---|---|
| US CL: | 435/7.1, 288, 805, 810; 436/518, 514, 544 546, 806, 807, 808, 809, 810; 422/56, 57, 58, 61 |

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